INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/21414

A. CLASSIFICATION OF SUBJECT MATTER
IPC: C12N 11/02(2006.01);A61F 2/06(2006.01)

USPC: 435/1.1,177;623/1.1;424/93.7
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/1.1, 177; 623/1.1; 424/93.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Medline, CAPlus (tissue engineering; artificial blood vessels)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	NIKLASON et al. Functional Arteries Grown In Vitro. Science. 16 April 1999, Vol. 284. ✓ pages 489-493.	1-13, 16, 17, 19, 26-39, 42, 43, 45, 50, 52-55, 57 --- 14, 15, 18, 20-25, 40, 41, 44, 46-49, 56, 58
X --- Y	RATCLIFFE et al. Tissue Engineering of Vascular Grafts, Matrix Biology, 2000, Vol. 19, pages 353-357.	1-9, 13, 14, 16, 17, 19, 26-34, 42, 43, 45, 50, 52-55, 57 --- 14, 15, 18, 20-25, 40, 41, 44, 46-49, 56, 58

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 2006 (16.02.2006)	16 MAY 2006
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Michael Wityshyn Telephone No. 571-272-1600

Janice Ford for

Form PCT/ISA/210 (second sheet) (April 2005)

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WEINBERG et al. A Blood Vessel Model Constructed from Collagen and Cultured Vascular Cells, Science. 24 January 1986, Vol. 231, pages 397-400.	22-25, 48, 49
Y	L'HEUREUX et al. In Vitro Construction of a Human Blood Vessel from Cultured Vascular Cells: A Morphologic Study. J Vasc Surg. 1993, Vol. 17, pages 499-509.	22-25, 48, 49
Y	TRANQUILLO, R.T., The Tissue-Engineered Small-Diameter Artery, Ann. N.Y.Acad. Sci. 2002, Vol. 961, pages 251-254, see whole document.	14, 15, 18, 21, 40, 41, 44, 47, 56, 58
Y	US 5,766,584 A (EDELMAN et al) 16 June 1998 (16.06.1998), whole document.	14, 15, 18, 21, 40, 41, 44, 47, 56, 58
Y	US 6,506,3987 B1 (TU et al) 14 January 2003 (14.01.2003), whole document. ✓	20, 46

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
WILLIAM F. LAWRENCE
FROMMER LAWRENCE & HAUG LLP
745 FIFTH AVENUE
NEW YORK, NY 10151

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Applicant's or agent's file reference 890003-2008	Date of mailing (*day/month/year*) 16 MAY 2006 FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/21414	01 July 2004 (01.07.2004)	01 July 2003 (01.07.2003)

International Patent Classification (IPC) or both national classification and IPC

IPC: C12N 11/02(2006.01);A61F 2/06(2006.01)
USPC: 435/1.1,177;623/1.1;424/93.7

Applicant

REGENTS OF THE UNIVERSITY OF MINNESOTA

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	21 February 2006 (21.02.2006)	Michael Wityshyn Telephone No. 571-272-1600

Form PCT/ISA/237 (cover sheet) (April 2005)

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

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Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO
Inventive step (IS)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO
Industrial applicability (IA)	Claims Please See Continuation Sheet	YES
	Claims Please See Continuation Sheet	NO

2. Citations and explanations:

Please See Continuation Sheet

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V.1. Reasoned Statements:
The opinion as to Novelty was positive (Yes)with respect to claims 15,18,20-25,40-41,44,46-49 and 51
The opinion as to Novelty was negative (No) with respect to claims 1-13, 14, 16, 17, 19, 26-39, 42, 43, 45, 50, 52-58
The opinion as to Inventive Step was positive (Yes)with respect to claims NONE
The opinion as to Inventive Step was negative(NO) with respect to claims 1-58
The opinion as to Industrial Applicability was positive (YES) with respect to claims 1-58
The opinion as to Industrial Applicability was negative(NO) with respect to claims NONE

V. 2. Citations and Explanations:
Claims 1-13, 16, 17, 19, 26-39, 42, 43, 45, 50, 52-58 lack novelty under PCT Article 33(2) as being anticipated by Niklason et al (Science, 1999).

Niklason et al teach a method of producing a tissue engineered blood vessel, comprising providing tubular biodegradable mesh polyglycolic acid (PGA) scaffolds (tubular support comprised of porous plastic); seeding the PGA tubular supports with smooth muscle cells (SMCs); culturing in culture medium for eight weeks to create a natural matrix circumferentially positioned around the PGA tubular support; then further seeding the matrix with endothelial cells (ECs); and finally culturing the vessel by perfusing culture medium through the lumen of the vessel so as to form a complete tissue engineered blood vessel (Pg. 490, col. 2-3 & Fig. 1). The final blood vessel comprises a matrix containing SMCs and ECs circumferentially positioned around a tubular mesh PGA support and culture medium, wherein the culture medium comprises mitogenic factors and attractant factors, such as platelet endothelial cell adhesion molecule (See Pg. 490, col. 3), that allow for the growth and survival of the cells, and wherein the mesh nature of the PGA support allows the mitogens and growth factors in the culture medium to perfuse through the support to the cells incorporated in the matrix. Niklason et al teach the SMCs and ECs can be obtained from bovine aorta (vascular tissue) (See Pg. 290, col. 2 & 3); please note all cells are derived from stem cells (Claims 1-13, 16, 17, 19, 26-39, 42, 43, 45, 53-55 and 57). In another embodiment Niklason et al create the tissue engineered blood vessels for transplantation into a test animal: Yucatan miniature swine (Claims 50 & 52); the tissue engineered blood vessels are produced as described above, using autologous SMCs and ECs obtained from the carotid artery (vascular tissue) of Yucatan miniature swine, in place of bovine aortic tissue-derived cells.

Claims 1-9, 13, 14, 16, 17, 19, 26-34, 42, 43, 45, 50, 52-55 and 57 lack novelty under PCT Article 33(2) as being anticipated by Ratcliffe et al (Matrix Biology, 2000).

Ratcliffe et al teach a method of producing a tissue engineered blood vessel, comprising providing a porous, tubular, non-biodegradable, polyurethane support scaffold (which applicant calls a tubular support comprised of porous plastic); seeding the

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

polyurethane scaffold with smooth muscle cells (SMCs); culturing in vitro so that the SMCs form a natural extracellular matrix comprising collagen, elastin and proteoglycan; then seeding endothelial cells (ECs) onto the luminal surface of the extracellular matrix created by the SMCs; culturing under fluid flow (through lumen) so as to form a complete tissue engineered blood vessel (See Pg. 356, col. 1). The final blood vessel comprises a matrix containing the SMCs and ECs as well as the naturally secreted proteins (collagen, elastin, and proteoglycans) circumferentially positioned around a tubular porous polyurethane support and culture medium, wherein the culture medium presumably comprises mitogenic factors and attractant factors to allow growth and adhesion of the cells within the graft (Claims 1-9, 13, 14, 16, 19, 26-34, 42, 43, 45, 53-55 and 57). Please note all cells are considered to be 'derived' from stem cells. Ratcliffe et al transplant the tissue engineered blood vessel into the carotid arteries of dogs (which applicant calls a subject in need thereof), and report the grafts remained patent for 4 weeks (Claims 50 and 52).

Claims 22-25, 48 and 49 lack an inventive step under PCT Article 33(3) as being obvious over Niklason et al (Science, 1999) and/or Ratcliffe et al (Matrix Biology, 2000), each in view of Weinberg et al (Science, 1986) and/or L'Heureux et al (Journal of Vascular Surgery, 1993).

As described above, both Niklason et al and Ratcliffe et al teach a tissue engineered blood vessel that is produced in vitro by seeding smooth muscle cells (SMCs) on a polymer support tube/scaffold; culturing the SMCs so as to allow formation of an extracellular matrix; followed by seeding endothelial cells (ECs) onto the extracellular matrix; and culturing by perfusing culture medium through the lumen of the artificial vessel to provide the appropriate mitogens and adhesion factors to allow growth and maintenance of the cells (See Niklason et al, Pg. 490, col. 2-3 & Ratcliffe et al Pg. 356, col. 1). While neither Niklason et al nor Ratcliffe et al teach applying an external layer of fibroblasts to the outside of the tissue engineered blood vessel, both Weinberg et al and L'Heureux et al teach application of a fibroblast layer on the exterior of artificial blood vessels. Weinberg et al use human skin fibroblasts to form an adventitia layer on their tissue engineered blood vessel, in order to better recreate natural blood vessel structure found in vivo (See Weinberg et al, Pg. 501, col. 1). Similarly, L'Heureux et al use human skin fibroblasts and formed a confluent sheet of fibroblasts that was wrapped around a mandrel, over a similar sheet of smooth muscle cells (See L'Heureux et al, Pg. 48, col. 2); L'Heureux et al report the fibroblast layer adhered to the smooth muscle layer to form a cohesive vessel. All fibroblasts are initial derived from stem cells. Therefore, it would have been obvious at the time of the invention to follow the teachings of Weinberg et al and L'Heureux et al to provide a layer of fibroblasts to the tissue engineered blood vessels of Niklason et al and/or Ratcliffe et al in order to better replicate the natural structure of a blood vessel in vivo; such a layer of fibroblasts on the exterior of the tissue engineered blood vessel is considered an outer casing surround the matrix (Claims 22-25, 48 and 49).

Claims 14, 15, 18, 21, 40, 41, 44, 47, 56 and 58 lack an inventive step under PCT Article 33(3) as being obvious over Niklason et al (Science, 1999) and/or Ratcliffe et al (Matrix Biology, 2000), each in view of Tranquillo (Ann. N.Y. Acad. Sci, 2002) and Edelman et al (US Patent 5,766,584).

While both Niklason et al and Ratcliffe et al teach a small diameter tissue engineered blood vessel and methods of forming the same, they each utilize a synthetic polymer as the support structure material, specifically Niklason et al use polyglycolic acid (See Niklason et al, Pg. 490, col. 2-3) and Ratcliffe et al teach use of polyurethane (See Ratcliffe et al, Pg. 356, col. 1). Tranquillo review tissue-engineered small-diameter artery grafts and teach that synthetic polymer scaffolds can be replaced with tubes formed of a biopolymer, such as Type I collagen, or more preferably fibrin gel, to produce a superior graft. Tranquillo teaches the biopolymer scaffolds are more preferable over synthetic polymer scaffolds because they can be subjected to mechanical constraint that results in circumferential alignment of fibrils and cells characteristic of the arterial media (See Tranquillo, Pg. 251 & 253). Tranquillo also teaches that when fibrin gel is used as the biopolymer, in place of collagen gel, smooth muscle cells exhibit enhanced collage synthesis in the presence of TGF-β (See Tranquillo, Pg. 253). Similarly, Edelman et al teach biodegradable materials are preferable, particularly biocompatible hydrogels that do not require subsequent removal before or after implantation; Edelman et al also teaches various natural polysaccharides can be used, though they don't specifically state agarose, this would be an obvious type of polysaccharide (See Edelman et al, col. 4, ln 56-67) (Claims 18 & 44). Edelman et al further teach various attachment factors can be added to the matrix to aid in cell adhesion and attachment, such factors include collagen, laminin, fibrin, fibronectin, basement membrane components (See Edelman et al, col. 5, ln 15-21) (Claims 21 & 47). Therefore, based on the teachings of Tranquillo and Edelman et al, it would have been obvious at the time the invention was made to modify the methods of Niklason et al and/or Ratcliffe et al to utilize biopolymer tubes, particular tubes of fibrin gel, as the scaffold support structure in place of the synthetic polymer scaffolds, so as to produce a tissue engineered blood vessel that is completely biocompatible (Claims 14, 15, 40, 41, 56 and 58).

Claims 20 and 46 lack an inventive step under PCT Article 33(3) as being obvious over Niklason et al (Science, 1999) and/or Ratcliffe et al (Matrix Biology, 2000), each in view of Tu et al (US Patent 6,506,398).

As described above, both Niklason et al and Ratcliffe et al teach a tissue engineered blood vessel that is produced in vitro by seeding smooth muscle cells (SMCs) on a polymer support tube/scaffold; culturing the SMCs so as to allow formation of an extracellular matrix; followed by seeding endothelial cells (ECs) onto the extracellular matrix; and culturing by perfusing culture medium through the lumen of the artificial vessel to provide the appropriate mitogens and adhesion factors to allow growth and maintenance of the cells (See Niklason et al, Pg. 490, col. 2-3 & Ratcliffe et al Pg. 356, col. 1). While Niklason et al mention including platelet endothelial adhesion molecule in the culture medium, both Niklason et al and Ratcliffe et al are silent on other specific factors included in the culture medium.

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Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

At the time of the invention it was generally known that culture media inherently contains necessary mitogens and adhesion factors to allow growth and survival of cells; however, Tu et al teach that vascular endothelial growth factors (VEGF) and platelet derived growth factors (PDGF) are particularly relevant in the culture of artificial blood vessels (See Tu et al, abstract). Tu et al teach that VEGF and PDGF act as site-specific angiogenesis factors that promote growth and survival of vascular endothelial cells (VEGF) and fibroblasts and smooth muscle cells (PDGF) (See Tu et al, col. 3, ln 47-58 & col. 4, ln 26-49); therefore, at the time the invention was made it would have been obvious to include both VEGF and PDGF in the culture medium used in the methods of Niklason et al and/or Ratcliffe et al.

Claims 1-58 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.